

# Phylogenetic analysis of three species of *Encarsia* (Hymenoptera: Aphelinidae) parasitizing *Bemisia tabaci* (Hemiptera: Aleyrodidae) in China based on their 28S rRNA gene

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**Abstract:** *Encarsia* Förster consists of important parasitoids of whitefly (*Bemisia tabaci*) pests, including *E. bimaculata*, *E. formosa* and *E. sophia*, the three most important aphelinid parasitoids in China. Eight populations of *Encarsia* from the South, Southeast, North and Southwest of China, as well as two populations from Malaysia and Egypt, respectively, were collected in the present study, and their inter-species phylogenetic relationships were analyzed based on 28S rRNA D2 and D3 expansion regions. The D2 and D3 regions were consistent with each other, confirmed a closer genetic relationship between *E. sophia* and *E. bimaculata* since they both belong to the *Encarsia strenus* species group, compared to those between these two species and *En. formosa*. Results of the genetic distance analysis using 28S rRNA D2 sequences revealed that there are certain genetic divergences within single species of the *Encarsia* parasitoids. The Guangzhou population of *Encarsia sophia* is more close to populations from Australia, Spain, Egypt and Ethiopia, but further from the population from Thailand. *E. bimaculata* populations from Sudan, Egypt and Guatemala as well as one population from Australia cluster together, while *E. formosa* Hengshui and Kunming populations cluster together with those from USA, UK and Greece, but are further from the Egypt population. The reasons for the inconsistency between the genetic and geographical distances of the *Encarsia* species are discussed.

**Key words:** Aphelinidae; *Encarsia*; 28S rRNA; genetic distance; phylogeny; *Bemisia tabaci*

## 1 INTRODUCTION

The sweetpotato whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) is a globally destructive agricultural pest (De Barro, 1995; Liu *et al.*, 2007; De Barro *et al.*, 2011). The first record of *B. tabaci* in China was in the late 1940's (Chou, 1949), but it was not considered as a serious pest until outbreaks in the mid-1990's due to the invasion of biotype B belonging to the Middle East-Asia Minor 1 genetic group (Qiu *et al.*, 2003, 2007a; Wu *et al.*, 2003) and then the subsequent invasion of the Q biotype belonging to the Mediterranean genetic group (Chu *et al.*, 2006). Since then, *B. tabaci* has caused serious damage to a wide range of vegetables, fiber and ornamental crops in more than 20 provinces across China (Zhang *et al.*, 2005; Li *et al.*, 2011).

One of the main challenges in *B. tabaci* management is that this pest continues to show an increasing level of resistance to a range of chemical insecticides, including nitenpyram, acetamiprid, imidacloprid and several pyrethroids (Morin *et al.*, 2002; Horowitz *et al.*, 2005; Byrne *et al.*, 2010; Feng *et al.*, 2010; Schuster *et al.*, 2010). Several recent studies in China have shown that most of the routine pesticides available have lost their effectiveness in *B. tabaci* control (He *et al.*, 2009; Qiu *et al.*, 2009; Wang *et al.*, 2009). Therefore, long-term sustainable management of *B. tabaci* requires an integrated approach in which biological control with natural enemies is one of the key components (De Barro *et al.*, 2000; Cuthbertson *et al.*, 2011).

During the last two decades, biological control agents of *B. tabaci*, including parasitoids, predators

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and both entomopathogenic fungi and nematodes as well as their associated technologies, have become the focus of research in *B. tabaci* management studies (Gerling *et al.*, 2001; Ren *et al.*, 2001; Cuthbertson *et al.*, 2008, 2011; Stansly and Naranjo, 2010). In China, 54 species of aphelinid parasitoids of *B. tabaci* have been recorded in a series of extensive field surveys, including 41 species of *Encarsia* and 12 species of *Eretmocerus* (Li *et al.*, 2011). Three species of *Encarsia*, for example, *E. bimaculata*, *E. formosa* and *E. sophia*, are the dominant species in China as revealed by extensive field surveys over the last decade (Ren *et al.*, 2001; Qiu *et al.*, 2004, 2007b; Li *et al.*, 2011).

Among the three dominant *Encarsia* species, *E. bimaculata* Heraty & Polaszek is a solitary, arrhenotokous, and heteronomous autoparasitoid. Mated females lay eggs internally in *B. tabaci* nymphs, and develop as primary parasitoids. Males develop as hyperparasitoids, either in females of their own species or in other primary aphelinid parasitoids (Antony *et al.*, 2004). This parasitoid is widely distributed in China, Australia, India, Thailand, Philippines, Sudan and the USA (Heraty and Polaszek, 2000). *E. formosa* is a cosmopolitan species (Polaszek *et al.*, 1992) and reproduces by microbe-induced thelytoky and has been successfully used in biological control of *Trialeurodes vaporariorum* on greenhouse crops in many countries (van Lenteren *et al.*, 1997; Hoddle *et al.*, 1998). *E. sophia* is also a cosmopolitan species found worldwide and a dominant parasitoid of *B. tabaci* (Kajita *et al.*, 1992; McAuslane *et al.*, 1993; Stansly *et al.*, 1997; Monti *et al.*, 2005) and *T. vaporariorum* (Gerling, 1983).

It is assumed that, when choosing natural enemy species as biological control agents, closely related species usually share the same habits and host preferences, and phylogenetic relationships might be used to assess desirable candidates (Babcock *et al.*, 2001). However, so far very little is known about these parasitoids in China, mainly because *Encarsia* populations in China have been rarely included in such studies, which might have delayed the accurate assessment and utilization of *Encarsia* parasitoids in whitefly control.

In the present study, the 28S rRNA D2 and D3 expansion regions of Chinese *E. bimaculata*, *E. formosa* and *E. sophia* populations were sequenced and analyzed with sequence data of other populations around the world to elucidate the relationships among them. The results are considered valuable in

evaluating the potential of local sources of these *Encarsia* parasitoids according to their relationship to the populations already reported, when choosing natural enemies in biological control programs.

## 2 MATERIALS AND METHODS

### 2.1 Parasitoid specimens

The study was largely based on the materials collected in 2006 – 2009 from the North, East, South and Southwest of China as well as from Kuala Lumpur, Malaysia (2007), and Egypt (2008 – 2009). All samples of the parasitoids were collected from the nymphs of whitefly in the fields, and each sample was noted with collecting date, locality and collector. The nymphs of *B. tabaci* parasitized by parasitoids were kept in emergence chambers and the emerged parasitoids were captured and transferred to 95% ethanol for storage. The whitefly species were identified either based on pupal morphology or by comparing their mitochondrial cytochrome oxidase I (mtCOI) sequence with that reported for *B. tabaci* (Qiu *et al.*, 2007b). The emerged parasitoids were identified by Dr. Huang Jian (Fujian Agriculture and Forestry University, China). In addition to *E. bimaculata*, *E. sophia* and *E. formosa*, several other species of *Encarsia* and *Eretmocerus* that emerged from the *B. tabaci* nymphs in our surveys were also included in the study. All voucher specimens of *Encarsia* and *Eretmocerus* are deposited in the Insect Museum of Department of Entomology, South China Agricultural University, Guangzhou, China.

### 2.2 DNA extraction and sequencing

The DNA extraction was done according to the description of De Barro and Driver (1997) with minor modifications. After washing by double distilled water to remove alcohol, individual parasitoids were homogenized in 200  $\mu$ L lysis buffer (1% SDS, 10 mmol/L Tris-HCl, pH 8.0, 25 mmol/L EDTA, 25 mmol/L NaCl, proteinase K 200 mg/mL) using a 0.5 mL micro centrifuge tube and micro pestle. The homogenate was incubated at 56°C for 2 – 3 h in a water bath and then at 95°C for 10 min to inactivate the Proteinase K. After incubation, samples were centrifuged for 1 min and directly used for PCR amplification or stored at –20°C for later use.

Sequences of the 28S rRNA D2 and D3 expansion regions were also obtained from 14 populations of 4 *Encarsia* species, 2 *Eretmocerus* species as well as from the outgroup *Amitus hesperidum* (Table 1), three individuals of each species were randomly selected for sequences

analysis. The PCR primers and amplification programs of 28S rRNA D2 and D3 expansion regions were described in De Barro *et al.* (2000). All PCRs were performed in a 25  $\mu$ L reaction volume and included 2.5 mmol/L  $MgCl_2$ , 200 mmol/L of each dNTP, 1  $\mu$ mol/L of each primer, 1 unit DNA Taq polymerase (Invitrogen, Guangzhou, China). Samples were amplified using a thermocycler (Bioer XP cycler, TC-XP-D). Afterwards, 5  $\mu$ L of PCR products were electrophoresed respectively using 1.0% agarose gels with  $1 \times$  TAE at 8 V/cm for 2 h, then the gels were dyed in 10 mg/mL ethidium bromide for 30 min. When bands with expected size were visible in the gels, the other 20  $\mu$ L of PCR products were used for sequencing.

### 2.3 Data analysis

Twenty five D2 and ten D3 sequences of *Encarsia* parasitoids were downloaded from GenBank and used as reference sequences (Table 2), and analyzed together with the sequences generated by us. All DNA sequences of 28S rRNA D2 and D3 regions of aphelinid parasitoids were first analyzed and aligned with DNASTar (Lasergene<sup>®</sup> v5.0) and ClustalX 1.83 (Thompson *et al.*, 1997). The phylogenetic relationships of different parasitoid populations were performed using NJ (Neighbor-joining) options available in MEGA 4.0. Genetic distances based on the D2 sequences were calculated with the Kimura 2-parameter model of MEGA 4.0.

**Table 1** Parasitoid species of *Bemisia tabaci* used in the phylogenetic analysis

Parasitoid species	Collecting locality	28S rDNA		GenBank accession no.
		D2	D3	
<i>Encarsia bimaculata</i>	Guangzhou, China	+		JF819982
<i>E. bimaculata</i>	Ismailia, Egypt	+		JF819983
<i>E. bimaculata</i>	Kuala Lumpur, Malaysia	+		JF819984
<i>Encarsia formosa</i>	Kunming, China	+		JF819985
<i>E. formosa</i>	Hengshui, China	+		JF819986
<i>Encarsia japonica</i>	Fuzhou, China	+		JF819987
<i>E. japonica</i>	Guangzhou, China	+		JF819988
<i>Encarsia sophia</i>	Guangzhou, China	+		JF819989
<i>E. sophia</i>	Ismailia, Egypt	+		JF819990
<i>E. sophia</i>	Kunming, China	+		JF819991
<i>Eretmocerus mundus</i>	Cairo, Egypt	+		
<i>E. mundus</i>	Ismailia, Egypt	+		JF819992
<i>Eretmocerus</i> sp. nr. <i>furushashii</i>	Guangzhou, China	+		
<i>Eretmocerus</i> sp. nr. <i>furushashii</i>	Sanya, China	+		
<i>Amitus hesperidum</i>	Guangzhou, China	+		JF819993
<i>E. bimaculata</i>	Guangzhou, China		+	JF819994
<i>E. bimaculata</i>	Ismailia, Egypt		+	JF819995
<i>E. bimaculata</i>	Kuala Lumpur, Malaysia		+	JF819996
<i>E. formosa</i>	Kunming, China		+	JF819997
<i>E. formosa</i>	Hengshui, China		+	JF819998
<i>E. japonica</i>	Fuzhou, China		+	JF819999
<i>E. japonica</i>	Guangzhou, China		+	JF820000
<i>E. sophia</i>	Guangzhou, China		+	JF820001
<i>E. sophia</i>	Ismailia, Egypt		+	JF820002
<i>E. sophia</i>	Kunming, China		+	JF820003
<i>Eretmocerus mundus</i>	Ismailia, Egypt		+	JF820004
<i>Eretmocerus</i> sp. nr. <i>furushashii</i>	Guangzhou, China		+	JF820005

+ : Target DNA sequences of the related parasitoid used in the phylogenetic analysis. The same below.

**Table 2 Parasitoid species used as references in the phylogenetic analysis**

Parasitoid species	Collecting locality	28S rDNA		GenBank accession no.
		D2	D3	
<i>Encarsia bimaculata</i>	India1	+		AF254214
<i>E. bimaculata</i>	Taiwan, China	+		AF254215
<i>E. bimaculata</i>	Australia1	+		AF254216
<i>E. bimaculata</i>	Australia2	+		AF254217
<i>E. bimaculata</i>	Sudan	+		AF254218
<i>E. bimaculata</i>	Guatemala	+		AF254219
<i>E. bimaculata</i>	India2	+		AF254220
<i>E. bimaculata</i>	Israel	+		AF254221
<i>E. bimaculata</i>	USA		+	AY599395
<i>E. bimaculata</i>	Australia		+	AY615748
<i>E. bimaculata</i>	Australia		+	AY615749
<i>Encarsia formosa</i>	Egypt	+		AF223372
<i>E. formosa</i>	Greece	+		AF223373
<i>E. formosa</i>	UK1	+		AF223374
<i>E. formosa</i>	UK2	+		AF223375
<i>E. formosa</i>	USA1	+		AF223377
<i>E. formosa</i>	USA2		+	AY599387
<i>E. formosa</i>	Australia		+	AY615759
<i>E. formosa</i>	USA3		+	AY615760
<i>Encarsia guadeloupae</i>	Tonga		+	AY615762
<i>Encarsia sophia</i>	Hawaii	+		AF254195
<i>E. sophia</i>	India	+		AF254196
<i>E. sophia</i>	Pakistan	+		AF254197
<i>E. sophia</i>	Ethiopia	+		AF254199
<i>E. sophia</i>	Florida, USA	+		AF254200
<i>E. sophia</i>	Spain1	+		AF254201
<i>E. sophia</i>	Malaysia	+		AF254202
<i>E. sophia</i>	Thailand	+		AF254203
<i>E. sophia</i>	Spain2	+		AF254204
<i>E. sophia</i>	Australia1	+		AF254205
<i>E. sophia</i>	Australia2	+		AF254206
<i>E. sophia</i>	Tahiti	+		AF254207
<i>E. sophia</i>	Ethiopia		+	AY599393
<i>E. sophia</i>	Australia		+	AY615743
<i>E. sophia</i>	Polynesia		+	AY615744

### 3 RESULTS

#### 3.1 Phylogeny of 28S rRNA D2 expansion segment

The amplified region of the 28S rRNA D2 expansion gene was about 590 bp in all of the

aphelinid parasitoids and sequences from the 2 – 3 replicated individuals of the same species were identical to each other. Because of the short fragment in some of the reference sequences retrieved from GenBank, all D2 sequences were trimmed after alignment to a final length of 459 bp.

The phylogenetic tree of these aphelinid



parasitoids based on 28S rRNA D2 expansion region is shown in Fig. 1. In the *E. sophia* clade, the populations from Hawaii (GenBank accession no.: AF254195) and Tahiti (GenBank accession no.: AF254207) were clustered into one subclade firstly, then they were clustered into one higher subclade with all other *E. sophia* populations including two from China. In the *E. bimaculata* clade, all the *E. bimaculata* populations except one from Australia (GenBank accession no.: AF254217) were clustered into one subclade. After that, the *E. sophia* clade and the *E. bimaculata* clade were clustered into one clade, which is a sister clade of *E. japonica*, the latter also included two *E. japonica* populations from Fuzhou and Guangzhou, China, respectively. Finally, the *E. sophia*, *E. bimaculata* and *E. japonica* formed a sister clade of the *E. formosa* clade. The phylogenetic tree also showed that *E. sophia* and *E. bimaculata* are sister species to each other, and they are more closely related to *E. japonica* than to *E. formosa*.

### 3.2 Phylogeny of 28S rRNA D3 expansion segment

The amplified region of the 28S rRNA D3 expansion gene was about 312 bp in all of the aphelinid parasitoids, and the same length of sequences were aligned together with some of the reference sequences from GenBank. The phylogenetic tree of these aphelinid parasitoids based on 28S rRNA D3 expansion region is shown in Fig. 2. In the NJ tree, three *E. bimaculata* populations from the USA (GenBank accession no.: AY599395), Australia (GenBank accession no.: AY615749) and Kuala Lumpur, Malaysia were firstly clustered together, followed with another Australian population (GenBank accession no.: AY615748). Afterwards, these four populations were clustered with the *E. bimaculata* population from Guangzhou, China, then with the *E. bimaculata* population from Ismailia, Egypt. Meanwhile, in the *E. sophia* clade, two populations from Africa, the Ethiopia population (GenBank accession no.: AY599393) and the population from Ismailia, Egypt were first clustered together, while the other four populations from Australia (GenBank accession no.: AY615743), Polynesia (GenBank accession no.: AY615744) and from Kunming and Guangzhou, China were clustered closely. One population of *Encarsia guadeloupae* from Tonga (GenBank accession no.: AY615762) was clustered as one sister subclade of five populations including two from China (Hengshui and Kunming). The phylogenetic patterns among the interspecies of

*Encarsia* parasitoids based on 28S rRNA D3 gene were consistent with that based on 28S rRNA D2 gene in Fig. 1.

### 3.3 Intra-species genetic distances of the three *Encarsia* species

The intraspecific genetic distances of the three *Encarsia* species were calculated using the 28S rRNA D2 sequences. Of all the 459 positions in the alignment, 11 positions varied, which were located between positions 16 and 437. The intra-species genetic distance of *E. sophia* is shown in Table 3. There are two base pair differences between the Guangzhou and Kunming populations of *E. sophia* (positions 173 and 235), both populations have the same genetic distance with the other *E. sophia* populations. For example, they have the closest genetic distance with the populations of Ismailia Egypt, Australia 1 and 2, Spain 1 and 2 as well as the population from Ethiopia (0.0022), then they have a relatively further genetic distance with those populations from India, Pakistan, Malaysia and Florida USA (0.0044), followed with larger distance with Hawaii and Tahiti populations (0.0066). The largest genetic distance was found between the *E. sophia* Guangzhou/ Kunming population and the Thailand population (0.0088).

In *E. bimaculata*, 8 positions varied in all the 459 positions of the 28S rRNA D2 region, and all of them were located between positions 70 and 369. The sequences of the Guangzhou, Ismailia Egypt, Australia 1, Sudan and Guatemala populations are identical to each other, thus they have the same genetic distance to the other *E. bimaculata* populations, being closer to the populations of Taiwan, India 1 and 2, Israel, Kuala Lumpur and Australia 1 (0.0022) than to the Australia 2 population (0.0066). Within *E. formosa*, the populations of Hengshui and Kunming from China, UK, USA (GenBank accession no. AF223377) and Greece are identical to each other, and have the same genetic distance to the population from Egypt (0.0022).

## 4 DISCUSSION

Several extensive surveys have reported 41 species of *Encarsia* parasitoids of whitefly pests in China (Ren *et al.*, 2001; Qiu *et al.*, 2004, 2007b; Li *et al.*, 2011). Although numerous studies have been reported on the morphological and/or molecular identification of *Encarsia* parasitoids (Polaszek *et al.*, 1992, 1999; Schauff and Evans, 1996; Huang and Polaszek, 1998; De Barro *et al.*, 2000; Babcock *et*

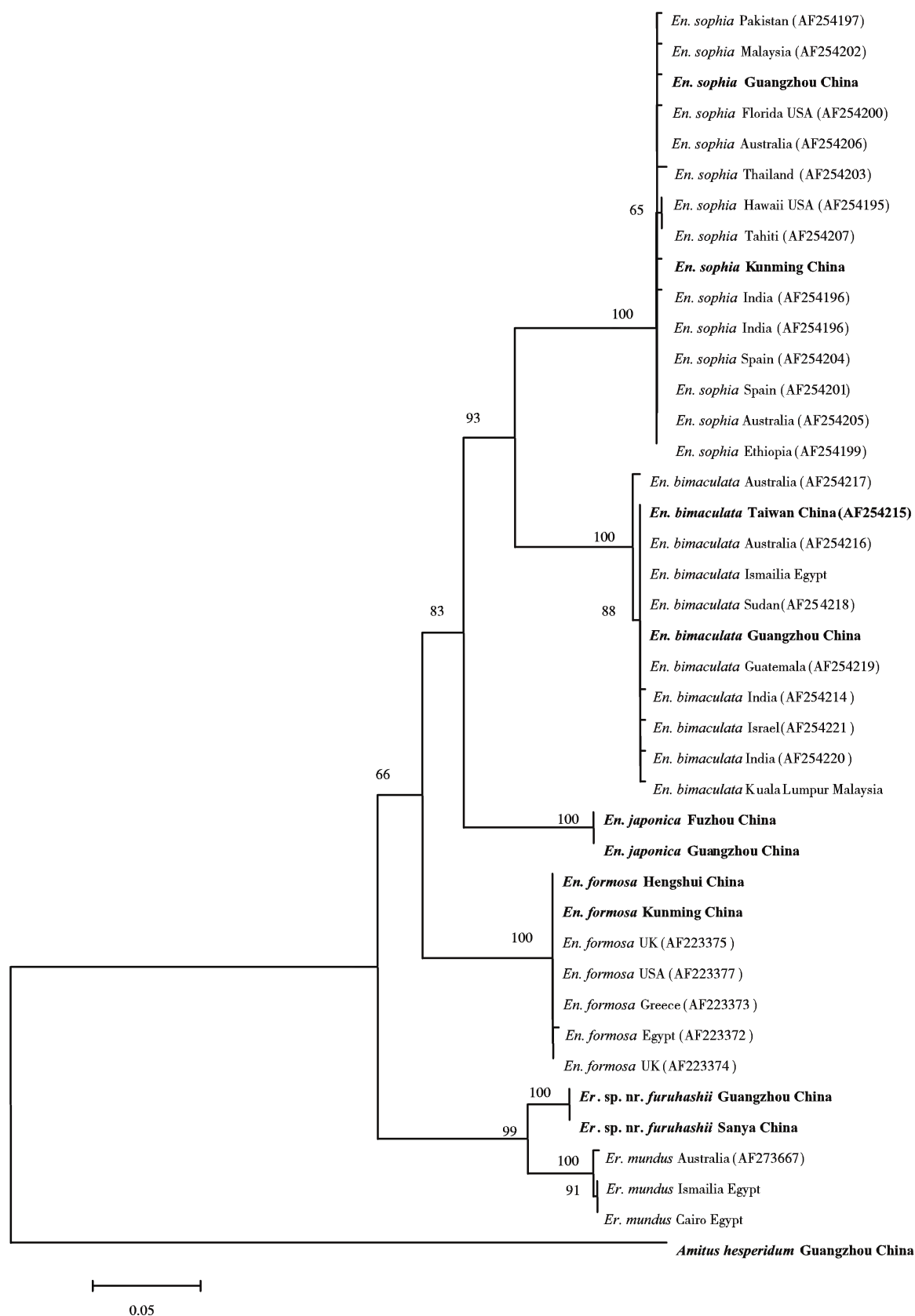


Fig. 1 The Neighbor-joining tree constructed with Kimura 2-parameter model showing the phylogenetic relationship of different aphelinid parasitoids based on their 28S rRNA D2 expansion region sequences

Values above the branches indicate the percent of 1 000 bootstraps. The same for Fig. 2.

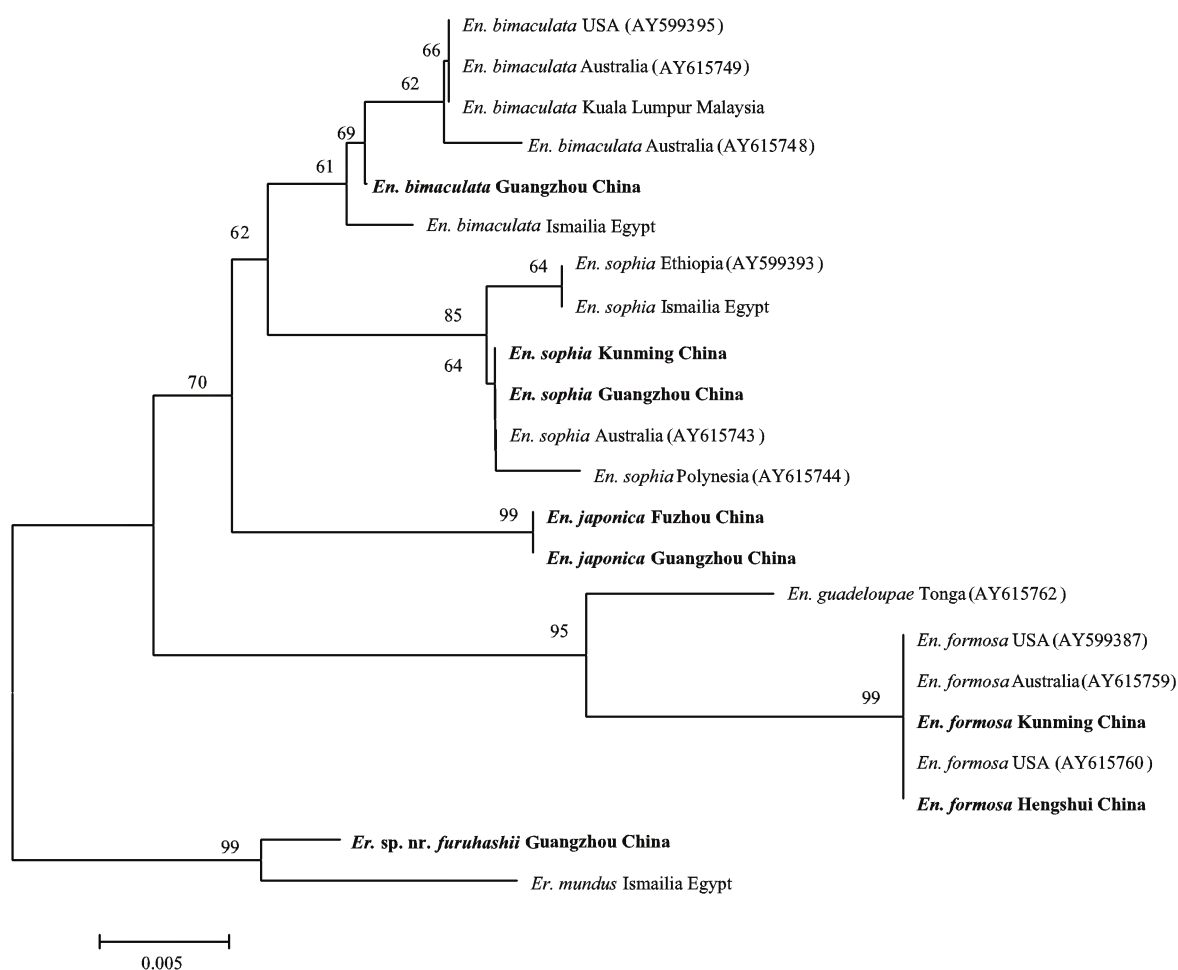


Fig. 2 The Neighbor-joining tree constructed with Kimura 2-parameter model showing the phylogenetic relationship of different aphelinid parasitoids based on their 28S rRNA D3 expansion region sequences

*al.*, 2001; Schmidt *et al.*, 2001; Pedata and Polaszek, 2003; Schmidt *et al.*, 2006; Qiu *et al.*, 2006, 2007b), few of them examined the phylogenetic relationship between the Chinese *Encarsia* populations with populations from other countries. This has prevented us from getting a relatively accurate evaluation of the biology of either the Chinese or introduced *Encarsia* populations without conducting extensive investigations.

In the current study, the intra-species phylogeny of *Encarsia* parasitoids based on 28S rRNA D2 and D3 expansion regions are consistent with each other, revealing the same phylogenetic pattern of *E. bimaculata*, *E. sophia*, *E. formosa* as well as *E. japonica*. The neighbor-joining tree based on D2 sequences revealed a range of genetic divergence of 0 – 17.88%, while the D3 phylogenetic tree revealed a range of genetic divergence of 0 – 4.64%, indicating that the 28S rRNA D3 expansion region is more conservative than the D2 region. Our result is consistent with the report of Schmidt *et al.* (2006) that D3 is the most conserved in the 28S rRNA D2, D3 and ITS-1 genes. Therefore, we subsequently used in

this study the 28S rRNA D2 gene segment to investigate the intraspecific genetic distance of the three dominant *Encarsia* species in China.

Our results on the intra-species genetic distance analysis of the three dominant *Encarsia* species indicated that there is genetic divergence between different populations within a single species, and the genetic distance is not consistent with their geographical ranges. For example, Guangzhou population of *E. sophia* is closer to those populations from Australia, Spain, Egypt and Ethiopia, but further to the populations from India, Pakistan and Thailand. Also, Guangzhou population of *E. bimaculata* is closer to those populations from Egypt and Sudan, but further to populations from Taiwan. Furthermore, even in Australia, two populations of *E. bimaculata* (GenBank accession no.: AF254216, AF254217) have different genetic distance from other *E. bimaculata* populations. The worldwide distribution of these commercial *Encarsia* populations through international trade and transportation of ornamentals with parasitized whitefly nymphs on the undersurface of plant leaves may be the reasons for the inconsistency in the genetic distance

Table 3 The genetic distance among Chinese *Encarsia* wasps and the other *Encarsia* populations worldwide based on their 28S rRNA D2 sequences

ESGuangzhou			ESKunming			ESIsmailia			EBGuangzhou			EBKualaLumpur			EBIsmailia			EFHengshui			EFKunming		
ESGuangzhou			0.0044			0.0022			EBGuangzhou			0.0022			0.0000			EFHengshui			0.0000		
ESKunming	0.0044					0.0022			EBKualaLumpur	0.0022					0.0022			EFKunming	0.0000				
ESIsmailia	0.0022	0.0022		0.0022					EBIsmailia	0.0000	0.0000	0.0022						EFUSA1	0.0000	0.0000			
ESHawaii	0.0066	0.0066	0.0066		0.0044				EBIndia1	0.0022		0.0044	0.0022		0.0022			EFUK2	0.0000	0.0000	0.0000		
ESTahiti	0.0066	0.0066	0.0066	0.0066	0.0044				EBTaiwan	0.0022	0.0022	0.0044	0.0044	0.0022	0.0022			EFUK1	0.0000	0.0000	0.0000		
ESIndia	0.0044	0.0044	0.0044	0.0044	0.0022				EBAustralia1	0.0000	0.0000	0.0022	0.0022	0.0022	0.0000			EFGreece	0.0000	0.0000	0.0000		
ESAustralia2	0.0022	0.0022	0.0022	0.0022	0.0000				EBSudan	0.0000	0.0000	0.0022	0.0022	0.0022	0.0000			EFEgypt	0.0044	0.0044	0.0044		
ESAustralia1	0.0022	0.0022	0.0022	0.0022	0.0000				EBGuatemala1	0.0000	0.0000	0.0022	0.0022	0.0022	0.0000								
ESSpain2	0.0022	0.0022	0.0022	0.0022	0.0000				EBIndia2	0.0022	0.0022	0.0044	0.0044	0.0022	0.0022								
ESSpain1	0.0022	0.0022	0.0022	0.0022	0.0000				EBIsrael	0.0022	0.0022	0.0044	0.0044	0.0022	0.0022								
ESMalaysia	0.0044	0.0044	0.0044	0.0044	0.0022				EBAustralia2	0.0066	0.0066	0.0088	0.0088	0.0066	0.0066								
ESEthiopia	0.0022	0.0022	0.0022	0.0022	0.0000																		
ESFlorida	0.0044	0.0044	0.0044	0.0044	0.0022																		
ESPakistan	0.0044	0.0044	0.0044	0.0044	0.0022																		
ESThailand	0.0088	0.0088	0.0088	0.0088	0.0066																		

ESGuangzhou; *Encarsia sophia* from Guangzhou; EBGGuangzhou; *E. bimaiculata* from Guangzhou; EFHengshui; *E. formosa* from Hengshui.



and geographical range of aphelinid parasitoids.

To summarize, our current result of genetic divergence within each species is more informative for evaluating the biology of these biocontrol agents. Our results also indicated that, compared to the tree of 28S rRNA D3 which has more resolution for the intra-specific relationship study of *Encarsia* parasitoids, the phylogenetic tree of 28S rRNA D2 region is less informative and not of much value to evaluate the biological characteristics of closely related aphelinid parasitoids, however, the D2 tree can be more useful in species identification of aphelinids since it provided highly robust support for each species. Furthermore, the reference data of 28S rRNA D3 region of *Encarsia* in GenBank are limited in numbers so far, more populations should be sampled for the systematic and comprehensive investigations that focus on the intra- and interspecific relationships of *Encarsia* wasps in future studies.

### References

- Antony B, Palaniswami MS, Kirk AA, Henneberry TJ, 2004. Development of *Encarsia bimaculata* (Heraty and Polaszek) (Hymenoptera: Aphelinidae) in *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) nymphs. *Biol. Control*, 30: 546–555.
- Babcock CS, Heraty JM, De Barro PJ, Driver F, Schmidt S, 2001. Preliminary phylogeny of *Encarsia* Förster (Hymenoptera: Aphelinidae) based on morphology and 28S rDNA. *Mol. Phylogenet. Evol.*, 18: 306–323.
- Byrne FJ, Oletting RD, Bethke JA, Green C, Chamberlin J, 2010. Understanding the dynamics of neonicotinoid activity in the management of *Bemisia tabaci* whiteflies on poinsettias. *Crop Prot.*, 29: 260–266.
- Chou I, 1949. Listo de la konataj Aleurodoj “Homoteroj” en cinio. *Entomol. Sin.*, 3: 1–18.
- Chu D, Zhang YJ, Brown JK, Cong B, Xu BY, Wu QJ, Zhu GR, 2006. The introduction of the exotic Q biotype of *Bemisia tabaci* from the Mediterranean region into China on ornamental crops. *Florida Entomol.*, 89: 168–174.
- Cuthbertson AGS, Blackburn LF, Eyre DP, Cannon RJC, Millar J, Northing P, 2011. *Bemisia tabaci*: the current situation in the UK and the prospect of developing strategies for eradication using entomopathogens. *Insect Science*, 18: 1–10.
- Cuthbertson AGS, Mathers JJ, Northing P, Prickett AJ, Walters KFA, 2008. The integrated use of chemical insecticides and the entomopathogenic nematode, *Steinernema carpocapsae* (Nematoda: Steinernematidae), for the control of sweetpotato whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Insect Science*, 15: 447–453.
- De Barro PJ, 1995. *Bemisia tabaci* Biotype B: A Review of Its Biology, Distribution and Control. 2nd ed. CSIRO Technical Paper, No. 36. 58 pp.
- De Barro PJ, Driver F, 1997. Use of RAPD PCR to distinguish the B biotype from other biotypes of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). *Aust. J. Entomol.*, 36: 149–152.
- De Barro PJ, Driver F, Naumann ID, Schmidt S, Clarke GM, Curran J, 2000. Descriptions of three species of *Eretmocerus* Haldeman (Hymenoptera: Aphelinidae) parasitising *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) in Australia based on morphological and molecular data. *Aust. J. Entomol.*, 39: 259–269.
- De Barro PJ, Liu SS, Boykin LM, Dinsdale AB, 2011. *Bemisia tabaci*: a statement of species status. *Annu. Rev. Entomol.*, 56: 1–19.
- Feng YT, Wu QJ, Wang SL, Chang XL, Xie W, Xu BY, Zhang YJ, 2010. Cross-resistance study and biochemical mechanisms of thiamethoxam resistance in B biotype *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Pest Manag. Sci.*, 66: 313–318.
- Gerling D, 1983. Observations of the biology and interrelationships of parasites attacking the greenhouse whitefly, *Trialeurodes vaporariorum* (West.) in Hawaii. *Proc. Hawaii Entomol. Soc.*, 24: 217–225.
- Gerling D, Alomar O, Arno J, 2001. Biological control of *Bemisia tabaci* using predators and parasitoids. *Crop Prot.*, 20: 779–799.
- He YX, Zhao JW, Huang J, Weng QY, Liang ZS, 2009. Frequency distribution of residual acetylcholinesterase activity in presence of inhibitor and its relationship with insecticide resistance in different populations of *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae). *Acta Entomol. Sin.*, 52: 1373–1378.
- Heraty JM, Polaszek A, 2000. Morphometric analysis and descriptions of selected species in the *Encarsia strenua* group (Hymenoptera: Aphelinidae). *J. Hymenopt. Res.*, 9(1): 142–169.
- Hoddle MS, van Driesche RG, Sanderson JP, 1998. Biology and use of the whitefly parasitoid *Encarsia formosa*. *Annu. Rev. Entomol.*, 43: 645–669.
- Horowitz AR, Kontsedalov S, Khasdan V, Ishaaya I, 2005. Biotypes B and Q of *Bemisia tabaci* and their relevance to neonicotinoid and pyriproxyfen resistance. *Arch. Insect Biochem. Physiol.*, 58: 216–225.
- Huang J, Polaszek A, 1998. A revision of the Chinese species of *Encarsia* Förster (Hymenoptera: Aphelinidae): parasitoids of whiteflies, scale insects and aphids (Hemiptera: Aleyrodidae, Diaspididae, Aphidoidea). *J. Nat. Hist.*, 32: 1825–1966.
- Kajita H, Samudra IM, Naito A, 1992. Parasitism of the tobacco whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae), by *Encarsia transvena* (Timberlake) (Hymenoptera: Aphelinidae) in Indonesia. *Appl. Entomol. Zool.*, 27: 468–470.
- Li SJ, Xue X, Ahmed MZ, Ren SX, Du YZ, Wu JH, Cuthbertson AGS, Qiu BL, 2011. Host plants and natural enemies of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in China. *Insect Sci.*, 18: 101–120.
- Liu SS, De Barro PJ, Xu J, Luan JB, Zang LS, Ruan YM, Wan FH, 2007. Asymmetric mating interactions drive widespread invasion and displacement in a whitefly. *Science*, 318: 1769–1772.
- McAuslane HJ, Johnson FA, Knauff DA, Colvin DL, 1993. Seasonal abundance and within-plant distribution of parasitoids of *Bemisia tabaci* (Homoptera: Aleyrodidae) in peanuts. *Environ. Entomol.*, 22: 1043–1050.
- Monti MM, Nappo AG, Giorgini M, 2005. Molecular characterization of closely related species in the parasitic genus *Encarsia* (Hymenoptera: Aphelinidae) based on the mitochondrial cytochrome oxidase subunit I gene. *Bull. Entomol. Res.*, 95: 401–408.
- Morin S, Williamson MS, Goodson SJ, Brown JK, Tabashnik BE, Dennehy T, 2002. Mutations in the *Bemisia tabaci* para sodium channel gene associated with resistance to a pyrethroid plus organophosphate mixture. *Insect Biochem. Mol. Biol.*, 32: 1781–1791.
- Pedata PA, Polaszek A, 2003. A revision of the *Encarsia longifasciata* species group (Hymenoptera: Aphelinidae). *Sys. Entomol.*, 28: 361–374.
- Polaszek A, Abd-Rabou S, Huang J, 1999. The Egyptian species of *Encarsia* (Hymenoptera: Aphelinidae): a preliminary review. *Zool. Med. (Leiden)*, 73: 161–163.
- Polaszek A, Evans GA, Bennet FD, 1992. *Encarsia* parasitoids of *Bemisia tabaci* (Hymenoptera: Aphelinidae, Homoptera: Aleyrodidae): a preliminary guide to identification. *Bull. Entomol. Res.*, 82: 375–392.
- Qiu BL, Coats SA, Ren SX, Idris AM, Xu CX, Brown JK, 2007a. Phylogenetic relationships of native and introduced *Bemisia tabaci* (Homoptera: Aleyrodidae) from China and India based on mtCOI DNA sequencing and host plant comparisons. *Prog. Nat. Sci.*, 17: 645–654.
- Qiu BL, De Barro PJ, He YR, Ren SX, 2007b. Suitability of *Bemisia tabaci* (Hemiptera: Aleyrodidae) instars for the parasitization by *Encarsia bimaculata* and *Eretmocerus* sp. nr. *furuhashii* (Hymenoptera: Aphelinidae) on glabrous and hirsute host plants.

- Biocontrol Sci. Technol., 17: 823–839.
- Qiu BL, De Barro PJ, Xu CX, Ren SX, 2006. Effect of temperature on the life history of *Encarsia bimaculata* (Hymenoptera: Aphelinidae), a parasitoid of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Eur. J. Entomol.*, 103: 787–792.
- Qiu BL, Liu L, Li XX, Mathur V, Qin ZQ, Ren SX, 2009. Genetic mutations associated with chemical resistance in the cytochrome P450 genes of invasive and native *Bemisia tabaci* (Hemiptera: Aleyrodidae) populations in China. *Insect Sci.*, 16: 237–245.
- Qiu BL, Ren SX, Lin L, Wang XM, 2004. Species and dynamics of aphelinid parasitoids of *Bemisia tabaci* in Guangdong. *Entomol. Knowl.*, 41: 333–335.
- Qiu BL, Ren SX, Wen SY, Mandour NS, 2003. Biotyping identification of the populations of *Bemisia tabaci* (Homoptera: Aleyrodidae) in China using RAPD-PCR. *Acta Entomol. Sin.*, 46: 605–608.
- Ren SX, Wang ZZ, Qiu BL, Xiao Y, 2001. The pest status of *Bemisia tabaci* in China and non-chemical control strategies. *Entomol. Sin.*, 18: 279–288.
- Schauff ME, Evans GA, 1996. A pictorial guide to the species of *Encarsia* (Hymenoptera: Aphelinidae) parasitic on whiteflies (Homoptera: Aleyrodidae) in North America. *Proc. Entomol. Soc. Wash.*, 98: 1–35.
- Schmidt S, Driver F, De Barro PJ, 2006. The phylogenetic characteristics of three different 28S rRNA gene regions in *Encarsia* (Insecta, Hymenoptera, Aphelinidae). *Org. Divers. Evol.*, 6: 127–139.
- Schmidt S, Naumann ID, De Barro PJ, 2001. *Encarsia* species (Hymenoptera: Aphelinidae) of Australia and the Pacific Islands attacking *Bemisia tabaci* and *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) – a pictorial key and description of four new species. *Bull. Entomol. Res.*, 91: 369–387.
- Schuster DJ, Mann RS, Toapanta M, Cordero R, Thompson S, Cyman S, Shurtleff A, Morris RF, 2010. Monitoring neonicotinoid resistance in biotype B of *Bemisia tabaci* in Florida. *Pest Manag. Sci.*, 66: 186–195.
- Stansly PA, Naranjo SE, 2010. *Bemisia*: Bionomics and Management of A Global Pest. Springer, New York, USA. 385–421.
- Stansly PA, Schuster DJ, Liu TX, 1997. Apparent parasitism of *Bemisia argentifolii* (Homoptera: Aleyrodidae) by Aphelinidae (Hymenoptera) on vegetable crops and associated weeds in South Florida. *Biol. Control*, 9: 49–57.
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG, 1997. The Clustal\_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.*, 25: 4876–4882.
- van Lenteren JC, Drost YC, van Roermund HJW, Postuma-Doodeman CJAM, 1997. Aphelinid parasitoids as sustainable biological control agents in greenhouses. *J. Appl. Entomol.*, 121: 473–485.
- Wang ZY, Yao MD, Wu YD, 2009. Cross-resistance, inheritance and biochemical mechanisms of imidacloprid resistance in B-biotype *Bemisia tabaci*. *Pest Manag. Sci.*, 65: 1189–1194.
- Wu XX, Li ZX, Hu DX, Shen ZR, 2003. Identification of Chinese populations of *Bemisia tabaci* (Gennadius) by analyzing the ribosomal *ITS1* sequence. *Prog. Nat. Sci.*, 13: 276–281.
- Zhang LP, Zhang YJ, Zhang WJ, Wu QJ, Xu BY, Chu D, 2005. Analysis of genetic diversity among different geographical populations and determination of biotypes of *Bemisia tabaci* in China. *J. Appl. Entomol.*, 129: 121–128.

## 中国寄生烟粉虱的三种恩角蚜小蜂 28S rRNA 系统发育分析

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**摘要:** 蚜小蜂 *Bemisia tabaci* 是烟粉虱的重要天敌, 其中双斑恩蚜小蜂 *Encarsia bimaculata*, 丽蚜小蜂 *E. formosa* 以及浅黄恩蚜小蜂 *E. sophia* 是国内烟粉虱寄生蜂 3 个优势种。本研究以采自中国华南、华东、华北、西南地区以及马来西亚、埃及的 *E. bimaculata*、*E. formosa* 和 *E. sophia* 3 个优势种的 8 个不同地理种群为研究对象, 对其 28S rRNA D2 和 D3 扩展区序列进行了测定和分析。结果表明: *Encarsia* 属的恩蚜小蜂其 28S rRNA D2 和 D3 序列在种间水平上高度保守; 与丽蚜小蜂相比, 双斑蚜小蜂与浅黄恩蚜小蜂在遗传关系上更为接近。依据 28S rRNA 和 D2 序列的系统发育分析结果显示, 同一种的蚜小蜂其种内也存在一定的遗传分化, 比如中国广东的浅黄恩蚜小蜂种群与澳大利亚、西班牙、埃及和埃塞俄比亚的浅黄恩蚜小蜂种群接近, 而与泰国的种群的亲缘关系则较远。在系统发育树上, 来自不同国家的(苏丹、埃及和危地马拉以及澳大利亚)的双斑蚜小蜂种群聚集在同一支上; 同时, 来自中国衡水和昆明的丽蚜小蜂种群也与来自美国的丽蚜小蜂种群聚集在一起, 却与埃及的种群相距较远。对造成这种同种寄生蜂不同种群之间在遗传距离和地理距离不对称的原因进行了探讨。

**关键词:** 蚜小蜂科; 恩角蚜小蜂; 28S rRNA; 遗传距离; 系统发育; 烟粉虱

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